I. CERTAIN FACTORS INVOLVED IN THE GROWTH OF *LACTOBACTILLUS CASEI*

II. THE LEUCINE AND ISOLEUCINE REQUIREMENTS OF MAN

BY

CHARLES HUBERT EADES, JR.

B. S., Southern Methodist University, 1938
A. M., The University of Texas, 1940

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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UNIVERSITY OF ILLINOIS, 1948

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I. CERTAIN FACTORS INVOLVED IN THE GROWTH OF *LACTOBACILLUS CASEI*

A. CALCIUM IONS AS A GROWTH STIMULANT FOR *LACTOBACILLUS CASEI*

Introduction

Investigations of the organic nutrient requirements of the lactic acid bacteria have been proceeding for some time, but slight attention has been devoted to the inorganic requirements. Chattaway, Happold, and Sandford (1) have presented evidence that calcium is needed for *maximum* acid production by *Lactobacillus casei.* McLeod and Snell (2) studied the inorganic erquirements of this organism and stated that 1 mg. of calcium in the form of a reagent grade salt per 10 ml. of medium showed only slight growth-promoting activity, and that this activity was lost after the salt had been recrystallized from distilled water. In connection with certain investigations described in Section I, Part B, of this thesis, it became necessary to formulate a medium which furnished, as nearly as possible, all of the inorganic components required by *Lactobacillus casei.* This led to a detailed study of the calcium requirement as outlined below.

Experimental

An amino acid type medium, modified from McMahan and Snell (3), was employed to test the growth effect of calcium on *Lactobacillus casei.* This element was found to enhance greatly the *early* growth of the organism. Experiments were performed to show that the effect was not just that of a bivalent ion. Manganese was found to influence the calcium effect, and a relationship between these two elements was established. By varying the proportions of calcium and man-

ganese in the medium, it has been possible to determine the optimum level of each for maximum early growth. As a result, a medium composed only of highly purified constituents of known composition has been designed for assay purposes. Standard microbiological techniques were employed throughout this work. The growth of the microorganism was measured by titration of the acid produced during 16 hours of incubation at 37°C.

Summary

Calcium has been shown to have a stimulatory effect on the rapid initial growth of *Lactobacillus casei* in a complete amino acid type medium. Five-tenths milligram of calcium per 10 ml. of final medium appears to exert the optimum effect.

The presence of manganese has been shown to have a definite relationship to the presence of calcium. For the latter to exert its optimum effect, 0.50 mg. of manganese per 10 ml. of medium appears to provide approximately the proper concentration.

A medium composed entirely of highly purified known constituents has been developed which supports rapid initial growth of *Lactobacillus casei*. The preparation and handling of the medium for assay work has been described.

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B. THE PRESENCE IN PROTEINS OF A SUBSTANCE WHICH STIMULATES THE GROWTH OF THE RAT AND *LACTOBACILLUS CASEI*

Introduction

For several years Womack and Rose (1) have been conducting investigations on an unknown substance present in proteins which stimulates the growth of the rat. This factor has been suspected of being related to the substance, "strepogenin," which has been reported by Sprince and Wooley (2,3). To determine if any relationship exists, materials were assayed for their activity as components of (1) rat diets and (2) media otherwise suitable for the growth of *Lactobacillus casei.*

Experimental

Many preparations such as tryptic digested fibrin, tryptic digested casein, acid hydrolyzed casein, Amigen, and Bacto-Peptone, were assayed for their activities with respect to both *Lactobacillus casei* and the rat. The medium and assay techniques developed in Section I, Part A, of this thesis were used with the microorganism. In the rat tests, the animals received an otherwise adequate diet containing a mixture of nineteen amino acids as the source of nitrogen. The supplements were supplied in the rat diets at a 5 per cent level. Levels of 1 to 10 mg. per culture were used with *Lactobacillus casei.* The growth of the microorganism was measured by titration of the acid produced in 16 hours. In the rat experiments, the stimulatory effect was determined by comparing the gains in weight of animals receiving the basal diet alone with the gains in weight of litter mates receiving the same diet supplemented with the protein materials. All tests of this nature were conducted for 28 day periods. In both species, the influence of tryptic digested fibrin was taken as the standard. The effects of other supplements were compared to this standard.

Summary

The results demonstrate that no clear-cut correlation exists between the effects of the supplements in question upon the two species of test organisms. Some materials showed comparable activity in both series of tests while others showed great divergence. In view of these findings, it seems unlikely that the substance, "strepogenin," which has been shown to stimulate the growth of *Lactobacillus casei,* is the only factor responsible for the growth acceleration of rats following the introduction of proteins into diets containing mixtures of amino acids as sources of nitrogen.

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II. THE LEUCINE AND ISOLEUCINE REQUIREMENTS OF MAN

Introduction

In 1942, studies on the amino acid requirements of man were undertaken in this laboratory, the criterion of nitrogen equilibrium being the basis of the investigations. Healthy young men (graduate students) were fed diets containing mixtures of highly purified amino acids as the chief sources of nitrogen. By removing each acid singly from the diet, its

effect upon nitrogen balance was determined (1-3). The amino acids essential for man were found to be tryptophan, lysine, phenylalanine, threonine, valine, leucine, isoleucine, and methionine. The quantitative requirements of these eight amino acids are being studied (4-9). In the present investigation, data on the human requirements for leucine and isoleucine are described.

Experimental

Male graduate students served as subjects. Their rations consisted of amino acids, urea, corn starch, sucrose, butter, corn oil, lemon juice, cellu flour, minerals and vitamins. The total daily caloric intake for each subject was kept constant throughout the experiment. The ratio of carbohydrate calories to fat calories was 2.6 to 1. Distilled water was taken ad libitum. Twenty-four hour samples of urine were collected and preserved with toluene, aliquots being analyzed for creatinine and total nitrogen. The feces for each experimental period were marked off by the ingestion of charcoal tablets and analyzed for total nitrogen. After a subject had attained positive nitrogen balance on a diet adequate in the essential amino acids, his intake of leucine or isoleucine, as the case might be, was progressively lowered in successive experimental periods until negative nitrogen balance was obtained. The total nitrogen intake was kept constant by adjusting the amount of glycine consumed to compensate for the removal of leucine or isoleucine. In this manner a level of leucine or isoleucine was determined which maintained the subject in consistent positive nitrogen balance, although an intake of 0.10 gm. less led to a negative balance, or fluctuations between positive and negative balances.

Summary

The minimum daily L-leucine requirement of three subjects was shown to be 0.50, 0.50, and 0.70 gm. respectively.

The minimum daily DL-isoleucine requirement of two subjects was shown to be 1.30 and 1.40 gm. respectively. Inasmuch as D-isoleucine is not utilized by man (9), the minimum daily L-isoleucine intake compatibile with consistent nitrogen balance in our subjects was 0.65 and 0.70 gm. respectively.

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VITA

The author was born on July 19, 1916 in Dallas, Texas. He received his elementary education in the public schools of Dallas and Highland Park, Texas, graduating with honors from the Highland Park High School in January, 1935. At that time he entered Southern Methodist University, receiving the Bachelor of Science degree with honors in June, 1938. The following September he entered the Graduate School of The University of Texas with an appointment as Tutor in the Department of Chemistry. He received the Master of Arts degree in June, 1940.

From June 1940 until June 1942 he was on the staff of the Paris Junior College, Paris, Texas, as Instructor of Sciences. In June 1942 he accepted a position with the Pan American Refining Corporation, Texas City, Texas, remaining there until he entered the Graduate College of the University of Illinois in October, 1945. He held an appointment as Teaching Assistant in Chemistry for the first semester, and an appointment as Special Research Assistant in Chemistry from that time until June, 1948. The author is a member of Phi Eta Sigma, Phi Lambda Upsilon, and Sigma Xi.